inflammation.[3] Fukui *et al.*[4] also reported that serum bilirubin levels were associated with microalbuminuria and subclinical atherosclerosis in patients with type 2 diabetes. This letter by Zelle *et al.*[1] further supported the relationship between serum bilirubin and diabetic nephropathy in a case–control study.

In the letter regarding the paper by Fukui *et al.*,[4] Kassimatis and Moutzouris[5] claimed that it was unwarranted to discuss the potential preventive and therapeutic application of bilirubin or its diagnostic utility as a new risk factor for diabetic nephropathy.[5] In fact, these association studies do not necessarily implicate the causative role of bilirubin in the development of diabetic nephropathy. Therefore, we investigated whether bilirubin may protect against diabetic nephropathy in experimental animals in our article.[2]

Prospective studies are needed to confirm its diagnostic utility as a risk factor for diabetic nephropathy. In addition, we agree that further intervention studies are needed to explore the possibilities of compounds with similar properties to bilirubin, which may represent a new class of therapeutic agents that could protect against the development of diabetic nephropathy.

1. Zelle DM, Deetman N, Alkhalaf A *et al.* Support for a protective effect of bilirubin on diabetic nephropathy in humans. *Kidney Int* 2011; **79**: 686.
2. Fujii M, Inoguchi T, Sasaki S *et al.* Bilirubin and biliverdin protect rodents against diabetic nephropathy by downregulating NAD(P)H oxidase. *Kidney Int* 2010; **78**: 905–919.
3. Inoguchi T, Sasaki S, Kobayashi K *et al.* Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes. *JAMA* 2007; **298**: 1398–1400.
4. Fukui M, Tanaka M, Shiraishi E *et al.* Relationship between serum bilirubin and albuminuria in patients with type 2 diabetes. *Kidney Int* 2008; **74**: 1197–1201.
5. Kassimatis TI, Moutzouris D-A. Bilirubin as a predictor of albuminuria and atherosclerosis in type 2 diabetic patients: misleading data. *Kidney Int* 2009; **75**: 862.

Masakazu Fujii[1] and Toyoshi Inoguchi[1,2]
[1]Department of Medicine and Regulatory Science, Graduate School of Medical Sciences, Kyushu University, Fykuoka, Japan and [2]Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan
Correspondence: *Toyoshi Inoguchi, Department of Medicine and Regulatory Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-Ku, Fukuoka 812-8582, Japan.*
E-mail: toyoshi@intmed3.med.kyushu-u.ac.jp

Kidney International (2011) **79,** 686–687; doi:10.1038/ki.2010.506

Circulating angiopoietin-1 could be confounded by *ex vivo* platelet activation

To the Editor: Reed *et al.*[1] recently reported the relationship between vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and renal as well as cardiovascular disease progression in patients with autosomal dominant polycystic kidney disease. Ang-1 and Ang-2 are antagonistic ligands of their common receptor Tie2. Unlike Ang-2, which is stored in endothelial cells, Ang-1 is not exclusively expressed by the vasculature; a high amount of Ang-1 is also found in platelets. Thus, falsely high Ang-1 serum levels may result from *ex vivo* activation of platelets upon clotting in serum tubes.[2] The same is probably true for vascular endothelial growth factor.[3]

Reed *et al.* found in the autosomal dominant polycystic kidney disease population slightly elevated Ang-2 and vascular endothelial growth factor (2–3 fold) serum levels compared with those earlier reported in healthy individuals. In contrast, mean Ang-1 levels in autosomal dominant polycystic kidney disease patients were 35.52 ± 21.03 ng/ml, which is more than 20 times higher than the levels we and others repeatedly detected in plasma from healthy subjects.[2] However, we had earlier published similar falsely high Ang-1 levels in patients' sera,[4] unaware of the *ex vivo* release out of platelets.

As Reed *et al.*[1] performed all measurements in patients' sera, one might question the validity and biological relevance behind these findings (in particular the Ang-1/Ang-2 ratio). In summary, it is possible that their results are confounded by platelet-derived Ang-1.

A reliable way to quantify Ang-1 in a compartment-specific way (i.e., the true circulatory fraction) would be highly desirable for the design of future studies.

1. Reed BY, Masoumi A, Elhassan E *et al.* Angiogenic growth factors correlate with disease severity in young patients with autosomal dominant polycystic kidney disease. *Kidney Int* 2011; **79**: 128–134.
2. Lukasz A, Hellpap J, Horn R *et al.* Circulating angiopoietin-1 and angiopoietin-2 in critically ill patients: development and clinical application of two new immunoassays. *Crit Care* 2008; **12**: R94.
3. Kusumanto YH, Dam WA, Hospers GA *et al.* Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003; **6**: 283–287.
4. David S, Kumpers P, Hellpap J *et al.* Angiopoietin 2 and cardiovascular disease in dialysis and kidney transplantation. *Am J Kidney Dis* 2009; **53**: 770–778.

Sascha David[1,2] and Philipp Kümpers[3]
[1]Center for Vascular Biology Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA; [2]Division of Nephrology, Department of Medicine, Medical School Hannover, Hannover, Germany and [3]Division of General Internal Medicine, Nephrology and Rheumatology, University Hospital Münster, Münster, Germany
Correspondence: *Sascha David, Division of Nephrology, Department of Medicine, Medical School Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany.*
E-mail: david.sascha@web.de and david.sascha@mh-hannover.de

Kidney International (2011) **79,** 687; doi:10.1038/ki.2010.504

The Authors Reply: We thank Drs David and Kümpers[1] for their interest in our recent *Kidney International* publication.[2] They point out that as angiopoietin-1 (Ang-1) is also found in platelets, serum levels of Ang-1 may be falsely high due to platelet activation. In our study, the most significant associations were between serum vascular endothelial growth factor (VEGF) level with renal and cardiac structure (left